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Genetic diversity of Central Javanese duck (Indonesia) based on Inter Simple Sequence Repeat markers

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Abstract. *Susanti R, Kartikasari AD, Sasi FA, Arlinda DD. 2022. Genetic diversity of Central Javanese duck (Indonesia) based on Inter Simple Sequence Repeat markers. Biodiversitas 23: 1807-1813.* Genetic resources of local livestock need to be documented and conserved to prevent genetic erosion of the germplasm. This study aimed to determine the genetic diversity of domestic ducks (*Anas platyrhynchos domesticus*) in Central Java, Indonesia, based on Inter Simple Sequence Repeat (ISSR) markers. There were seven types of Central Javanese ducks used in this research. DNA was extracted from feathers samples. To give rise to ISSR profile of duck DNA, 5 different ISSR markers were used. Annealing temperature used in PCR was the result of the optimization. The ISSR-PCR results showed that all primers (100%) were polymorphic. From a total of five ISSR primers, resulted in 44 obvious bands in all duck samples, with 41 (91.78%) bands being polymorphic. The quantity of alleles was numerous from 7-14 polymorphic loci, the mean quantity of alleles according to locus was 8.8, and the average PIC score for the Central Javanese duck was 0.777. The relationship investigation showed that the Central Javanese local duck was clustered into two primary offshoots, one heading to Pengging duck breeds and another one consisting of all other Central Javanese local duck. The genetic diversity among Central Javanese local duck based on ISSR primer was high. The ISSR loci were polymorphic for Central Javanese duck and may be useful for study of genetic diversity and genome evolution of Central Javanese ducks in the future.

Keywords: Allele, Central Java, heterozygosity, ISSR-PCR, polymorphic information content

INTRODUCTION

The conservation of genetic diversity has arisen in recent times since the presence of a big gene pool is significant for the subsequent reproductive capability and for the construction of sustainable animal production system. Comprehensive people awareness of the available genetic diversity is an initial prerequisite for conservation and utilization of domestic animal biodiversity. Sustaining the genetic variability of livestock species is one of the most important concerns for husbandry guidelines and critical pace to secure the stocks of endangered domestic animals.

The domestic duck (*Anas platyrhynchos domesticus*) is one of the poultry species that has commercial and ecological value, as well as social function, especially in Malaysia, Indonesia, Vietnam, China, and others (FAO 2015). There are many types of local ducks in Indonesia, such as Tegal, Magelang, and Mojosari ducks in Java, Bali ducks in Bali, and Alabio ducks in Kalimantan (Ismoyowati and Purwantini 2010). Indonesian ducks showed variations in feather color, anatomic size, and eggs production. For reproduction purposes, it is critical to research genetic diversity using a molecular approach. Variations in certain genes are involved in the phenotypic traits of reproductive efficiency. In ducks, the diversity of X-collagen (COLX), prolactin (PRL) and ovomucoid genes, respectively, were associated with egg performance (Li et al. 2006), egg weight (Chang et al. 2012), and egg hatchability (Lin et al. 2016). However, there is limited genetic documentation about domestic duck populations in Indonesia. Most research on ducks in Indonesia focus on their morphology and productivity (Ismoyowati et al. 2012), phenotypic characterization (Tamzil and Indarsih 2017; Maharani et al. 2019), and breeding or feeding (Suci et al. 2017; Ismoyowati et al. 2018; Susanti et al. 2020; Susanti et al. 2021a).

In Indonesia, genetic diversity of duck turned into finished through numerous molecular techniques which include microsatellite (Susanti et al. 2021b) and mitochondrial DNA (Susanti et al. 2017; Susanti et al. 2018), but there are no reports concerning the use of ISSR marker on domesticated duck. Inter-simple sequence repeats (ISSRs) are domains inside the genome clamped by microsatellite sequences (simple sequence repeats; SSRs). The ISSR is a polymerase chain reaction (PCR) - primarily based technique, using a single primer of microsatellite (repeat of dinucleotide, trinucleotide, tetranucleotide, or pentanucleotide). ISSR has several advantages, because the protocol is relatively simple, rapid, low-cost (Dogan et al. 2015) and does not require sequence information for primer design (Son et al. 2012). ISSRs can also be utilized for gene and population genetic mapping, as well as to recognize the breeds. Identification of genetic diversity on the duck population in Central Java using the ISSR marker has not been documented. Therefore, this study aimed to

determine the genetic diversity of domestic ducks in Central Java, Indonesia primarily based on ISSR markers.

MATERIALS AND METHODS

Sample preliminaries

This study used 35 duck samples, consisting of seven types of ducks from Central Java, Indonesia namely Magelang duck (IM), Peking duck (IK), Pengging duck (IG), Tegal Branjangan duck (ITB), Tegal Distance duck (ITJ), Tegal Blorong duck (ITL), and Tegal Lemahan duck (ITP). All types consisted of five ducks, respectively. Samples had been taken from Balai Pembibitan dan Budidaya Ternak Non Ruminansia (BPBTNR) Dinas Kesehatan Hewan (Dinkeswan) Satuan Kerja Itik Banyubiru, Semarang District (Non-Ruminant Cultivation and Livestock Breeding Center, Animal Health Service, Banyubiru Duck Work Unit, Semarang District), Central Java, Indonesia. Samples were selected randomly with the standards for sampling (inclusion): 1. Ducks which included seven varieties of ducks from Central Java, 2. As a minimum three month old female (hen) ducks, and 3. Healthy individuals.

For ISSR-PCR analysis, the duck DNA was taken from plumage feathers on the right and left wings. In this DNA isolation, the parts of duck feather samples were calamus and rachis, including its proximal umbilicus because these parts were filled with marrow. Sample preparation for DNA isolation from feathers was carried out according to manual protocol from DNA extraction kit manufacturer.

DNA extraction

DNA was extracted from feathers samples using the manual protocol of gSYNC™ DNA Extraction Kit (Geneaid Biotech Ltd., New Taipei City, Taiwan). The characteristic and mass of isolated DNA had been decided by 0.8% agarose (Sigma) gel electrophoresis and a spectrophotometer utilizing the NanoDrop 2000C (Thermo Scientific, Waltham, MA, USA). Absorbance ratio value of 260/280 nm from the isolated duck DNA in this study was in the range of 1.78-1.89. This ratio value is in the range of pure DNA values (1.8-2.0), so it can be continued for ISSR-PCR analysis. DNA isolation was declared pure if the absorbance ratio was in the range of 1.8-2.0.

ISSR-PCR analysis

To give rise to ISSR profile of duck DNA, five dissimilar ISSR markers (Tunca et al. 2015) from 1st BASE (Apical Scientific Sdn. Bhd., Selangor, Malaysia) were used in this study (Table 1). Amplification was carried out employing KAPA2G HotStart ReadyMix Kit (Kapa Biosystems, Massachusetts) with the composition of 12.5 µL ready mix, 1.5 µL primer (10 ng/µL concentration), 9.0 µL of ddH_2O, and 2.0 µL of DNA samples (50 ng/µL concentration). GeneAmpR PCR system thermocycler 2400 (PerkinElmer, Inc. Massachusetts, USA) was used in this PCR technique. PCR procedure steps had been pre-denaturation at 94°C for 5 minutes, denaturation at 94°C for 1 minute, annealing at 50.0-56.0°C for 1 minute, elongation at 72°C for 1 minute, and an extended elongation at 72°C for 5 minutes. The cycle was repeated 35 times. Annealing temperature used in this research was the result of the optimization (Table 1). The amplification results were dissociated with the molecular weight marker (100bp GeneRuler, Fermentas, Canada) on 1.2% agarose (Sigma) gel in TBE (Tris/Borate/EDTA) solution and pigmented with ethidium bromide. The molecular weight of every band or allele that appeared became measured or predicted with Alpha Imager software program from Alpha Imager®EP (Cell Biosciences Inc., Santa Clara, CA).

Data analysis

The presence of the clear visible ISSR band was given a score of 1, while a score of 0 denoted no or weak bands produced. Heterozygosity (Hi) for every ISSR marker became calculated utilizing a formulation as stated by way of Nei (1987):

$$H_i = \frac{2N}{2N-1}\left[1 - \sum_{j=1}^{l} Pj^2\right]$$

Where: *Pj* is frequency of the j^{th} allele at the i^{th} locus; *k* is alleles quantity in population; and *N* is individuals' quantity.

The polymorphic information content (PIC) score turned into calculated using a formulation as stated by way of Botstein et al. (1980):

$$\text{PIC} = 1 - \sum_{i=1} Pi^2 - 2\sum_{j=i}\sum_{i=j+1} Pi^2\, Pj^2$$

Where: *Pi* and *Pj* are the frequencies of i^{th} and j^{th} allele, respectively, at a locus with *n* alleles in a population.

Relationships were analyzed using the unweighted pair group technique using the mean value with numerical taxonomy and multivariate analysis system (NTSYSpc) version 2.01 (Rohlf 1998). The phylogenetic tree became constructed following the software's protocol. Genetic similarity coefficients between individuals had been processed utilizing SIMQUAL methods and calculated based totally on the Dice coefficient from Sneath and Sokal (1973).

RESULTS AND DISCUSSION

ISSR-PCR in this research was done with the optimized annealing temperature. The annealing temperature used in this study was a little different with the Anatolian ducks (Tunca et al. 2015) and chicken (*Gallus gallus domesticus*) (Tunca and Taskin 2016) (Table 1). Representative ISSR fingerprints acquired with primer AC8CA and GA8CC are shown in Figure 1.

Table 1. The use of ISSR primers with optimized annealing temperature from previous research

Primer	Repeat type	Nucleotide sequence (5´ to 3´)	Annealing temperature (°C)		
			Modification in this study	Tunca et al. (2015)	Tunca and Taskin (2016)
ISSR1	(CA)8G	CACACACACACACACAG	50.1	50.0	55.0
ISSR2	(AG)8G	AGAGAGAGAGAGAGAGG	55.1	55.0	54.0
ISSR3	(AC)8CA	ACACACACACACACACCA	54.3	54.0	55.0
ISSR4	(GA)8GG	GAGAGAGAGAGAGAGAGG	56.1	56.0	-
ISSR5	(GA)8CC	GAGAGAGAGAGAGAGACC	53.4	54.0	-

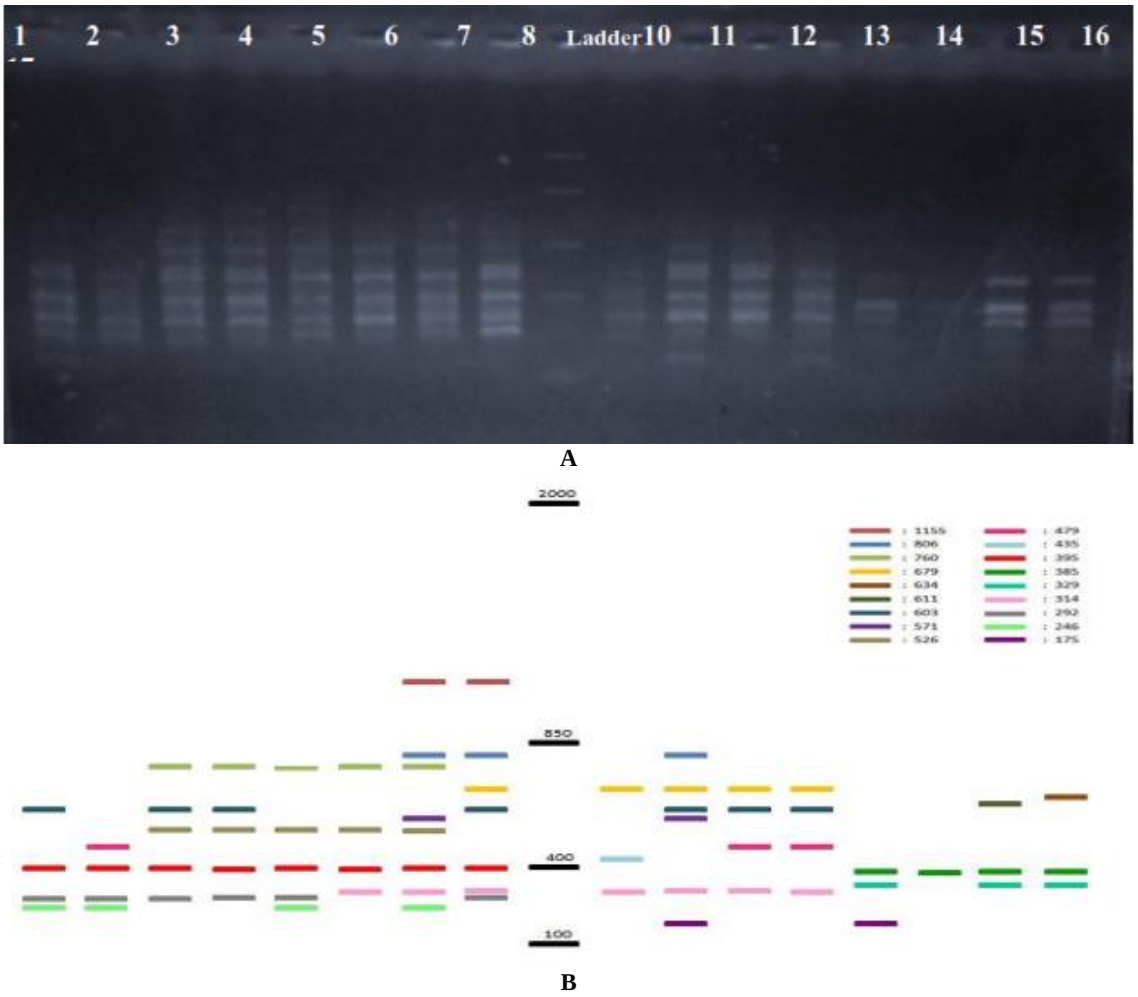

Figure 1. Allele visualisation of ISSR-PCR where line 1-2 represent Tegal Blorong duck (ITL), line 3-7 for Tegal Lemahan duck (ITP), line 8 and 10-13 for Magelang duck (IM) with (AC)8CA primer, line 14-17 for Tegal Branjangan duck (ITB) with (GA)8CC primer, line 9 for ladder (100bp) on (A) agarose gel electrophoresis and (B) zymogram. The colored bands indicate alleles positions on the agarose gel and different colors indicate different alleles and size lengths

ISSR polymorphism

The ISSR-PCR outcomes confirmed that all primers (100%) were polymorphic (Table 2). A total of five ISSR primers, resulted in 44 obvious bands in all duck samples, with 41 (91.78%) polymorphic bands, indicating a great genetic diversity. The three monomorphic bands (only found in 1 sample of ducks) were 866 bp band on Tegal Branjangan 1 duck (ITB1), 1014 bp band on Tegal Blorong 5 duck (ITL5), and 492 bp band on Tegal Jarakan 1 duck (ITJ1). Previous research indicated that ISSR-PCR analysis on chicken DNA with 15 primers yielded 87 bands (Tunca and Taskin 2016). Five primers for ISSR analysis on mink (*Neovison vison*) DNA yielded 35 bands and 85.71% of the bands were polymorphic (Hong-yan et al. 2013). In Anatolian duck DNA, five primers used for ISSR-PCR analysis were able to produce 73 bands and 72 of them (98.63%) were polymorphic (Tunca et al. 2015). In rabbit DNA, the six ISSR primers were able to produce 141 bands

and 87 (61.7%) bands were polymorphic (El-Sabrout and Aggag 2015). While in the population of cattle (*Bos taurus*), sheep (*Ovis aries*), and goats (*Capra hircus*), the two ISSR primers used were able to produce 60 bands, with polymorphic loci ranging from 26.67% to 81.67%. The number of polymorphic loci in cattle varied from 23-49 bands, in goats 14-20 bands and in sheep 33-34 bands (Askari et al. 2011).

The number of bands resulting from ISSR-PCR was between 7 to 14 bands per primer, with a mean of 8.8 bands per primer. The highest amount of ISSR bands (14 bands) was produced by primers (AC)8CA, while the lowest was produced by primers (AG)8G and (GA)8GG with seven bands. The approximate range of band size was 158-1271bp (Table 2). The number of bands of each ISSR primer in this study is in contrast to the number of bands in Anatolian ducks (Tunca et al. 2015). In this research, (AG)8G primer that produced seven bands for central Javanese local ducks, only showed five bands for Anatolian ducks (Tunca et al. 2015), and showed the different number for chicken (4 bands) (Tunca and Taskin 2016) (Table 3). Based on the comparison of the band's number formed from each ISSR primer (Table 3), it appeared that the local Central Javanese ducks had the highest diversity compared to Anatolian duck and chicken. Anatolian duck is a domestic duck in the central Anatolian region of Turkey. This study also conducted an analysis of the diversity of ducks in the Central Java region.

The number of different ISSR-PCR band products can be caused by insertion or deletion or the disappearance of primer attachment sites, resulting in distinct binding motifs. The ISSR marker shows better reproducibility than RAPD, because the primary sequence used in the ISSR is longer, so the annealing temperature is also higher. The degree of polymorphism of the ISSR-PCR genetic marker is largely determined by the type of primer used. One of the main advantages of ISSR analysis is that ISSR uses only a single primer (not a pair). Another advantage is that the ISSR primer is a simple repeating sequence and does not require a special primer design (Son et al. 2012; Parthasarathy et al. 2013). Also, ISSR analysis doesn't require use of radioactivity. ISSR is sensitive, reliable, and incorporates more information than other dominant molecular techniques (Gradzielewska 2011; Parthasarathy et al. 2013).

Genetic diversity

The genetic diversities amongst Central Javanese duck are shown in Table 4. Commonly, 44 alleles had been recognized from five polymorphic loci. The alleles were numerous from 7-14 polymorphic loci, in which the maximum considerable alleles were determined in (AC)8CA locus and the minimum considerable was in (AG)8G and (GA)8GG locus. Moreover, the mean quantity of alleles according to locus was 8.8 where the average PIC score for the Central Javanese duck was 0.777. The PIC scores for five polymorphic loci studied had been high, ranging from 0.646 to 0.848 for (AG)8G and (AC)8CA, respectively. There were five loci (100%) with PIC score greater than 0.500 (Table 4). PIC score is a proper indicator of genetic variety assessment where a PIC score higher than 0.500 suggests a high genetic diversity. Otherwise, PIC score lower than 0.25 suggests low diversity, and range amongst 0.25 and 0.5 suggests medium diversity (Botstein et al. 1980). Based on this data, all polymorphic ISSR loci had sufficient discrimination functionality to distinguish individuals and breeds in population.

Table 2. The number and size of the amplified bands with 5 types of ISSR primers and their polymorphism

Primer	Quantity of loci	Band size (bp)	Quantity of polymorphic loci	Percentage of polymorphic loci (%)
(CA)8G	8	357-1271	7	87.5%
AG)8G	7	262-1014	5	71.4%
(AC)8CA	14	175-1155	14	100%
(GA)8GG	7	158-677	7	100%
(GA)8CC	8	273-634	8	100%
Total	44	158-1271	41	91.78%

Table 3. Number of bands per primer on Central Javanese local duck, Anatolian duck, and chicken

Primer	Number of loci		
	Central Javanese duck (this study)	Anatolian duck (Tunca et al. 2015)	Chicken (Tuncaand Taskin 2016)
(CA)8G	8	6	7
(AG)8G	7	5	4
(AC)8CA	14	4	4
(GA)8GG	7	5	no data
(GA)8CC	8	6	no data
Total	44	26	

The predicted heterozygosity scores for diverse loci were about 0.655 to 0.884 with an average score of 0.796 (Table 4). In a previous study, it was shown that the average genetic diversity of Nei (H) (Nei 1987) varied in various animals, i.e., 0.0655 in goats, 0.2076 in sheep (Askari et al. 2011), and 0.118 in chicken (Tunca and Taskin 2016). Values of the effective quantity of alleles (Ne) in this duck were about 2.8 to 6.6, higher than goat (1.1085 ± 0.2624), sheep (1.3673±0.4050) (Askari et al. 2011), chicken (0.157), mink (1.5180) (Hong-yan et al. 2013), and Anatolian duck (1.26) (Tunca et al. 2015). ISSR is a molecular marker that has been proven to be effective and low-price to evaluate genetic variety that is broadly utilized in species recognition and genetic correlation investigation (Dogan et al. 2015).

Allele frequency for numerous loci ranged among 0.011 and 0.421. From 44 alleles, there were no alleles that had scores higher than 0.5 (Table 4). It can be seen in Table 4 that some alleles had very high frequencies. At least 17 out of 44 alleles (38.6%) were polymorphic with a frequency of over than 10%. According to Rajkumar et al. (2008), alleles that have a frequency of about 10% are very suitable to be used to characterize certain populations. This advised that the alleles can be used as characterization markers for the Central Javanese duck population as dissimilar populations with the other genuine Indonesian ducks.

Table 4. ISSR markers traits in Central Javanese duck

Primer	Fragment (bp)	Hi	PIC	NA	NE	Allele	Size (bp)	Frequency
(CA)8G	357-1271	0.808	0.796	8	4.9	A	1271	0.056
						B	866*	0.011
						C	745	0.033
						D	640	0.167
						E	556	0.222
						F	460	0.278
						G	391	0.022
						H	357	0.211
(AG)8G	262-1014	0.655	0.646	7	2.8	A	1014*	0.013
						B	547	0.092
						C	492*	0.026
						D	409	0.013
						E	371	0.421
						F	317	0.026
						G	262	0.408
(AC)8CA	175-1155	0.860	0.848	14	6.6	A	1155	0.013
						B	806	0.047
						C	760	0.033
						D	679	0.073
						E	603	0.120
						F	571	0.080
						G	526	0.040
						H	479	0.027
						I	435	0.127
						J	395	0.093
						K	314	0.180
						L	292	0.100
						M	246	0.047
						N	175	0.020
(GA)8GG	158-677	0.773	0.762	7	4.2	A	677	0.044
						B	409	0.044
						C	372	0.338
						D	301	0.074
						E	277	0.309
						F	214	0.088
						G	158	0.103
(GA)8CC	273-634	0.884	0.832	8	5.9	A	634	0.076
						B	611	0.044
						C	572	0.054
						D	520	0.076
						E	473	0.141
						F	385	0.228
						G	329	0.250
						H	273	0.130
Average		0.796	0.777	8.8	4.88			

Note: *Monomorphic band

Similarly, analysis confirmed the presence of particular alleles in Central Javanese duck breeds. Identification yields revealed the emergence of three specific alleles in the Tegal Lemahan duck breed (ITP) and one specific allele in Magelang duck (IM) (Table 5). Specific microsatellite allele was also shown in Central Javanese duck breeds, i.e., Tegal Branjangan duck (ITB), Peking duck (IK), Pengging duck (IG), and Tegal Jarakan duck (ITJ) (Susanti et al. 2021b). The appearance of these particular alleles issued a perception that these strains had special phenotypic properties in view that molecular markers are regularly positioned close to genes. Forty-four alleles had been detected from five ISSR loci inside the seven breeds. The average quantity of alleles according to breed of duck ranged between 2.52 to 3.12 (Table 6).

Dendrogram was built to support the similarity data among seven breeds/types of Central Javanese duck. Cluster investigation exhibited two primary offshoots, one headed to Pengging duck breed, and the other clustered into two subdivisions; one subdivision consisted of Tegal Branjangan, Peking, and Tegal Lemahan duck breeds, and the other consisted of the Tegal Jarakan, Tegal Blorong, and Magelang duck breeds. The Pengging duck comes from the Pengging area, Banyudono District, Boyolali Regency. The Peking duck is a descendant of the Mallard duck (*A. platyrhynchos*), imported from China to Indonesia and spread in several regions, one of which is in Central Java. Peking ducks can adapt to Indonesian environmental conditions. Based on the ISSR marker, the ducks were not grouped according to "sub-regional origin". Tegal ducks do not clump together in the same cluster. Peking ducks and Magelang ducks are grouped into subdivisions with Tegal ducks. This indicates that groups of ducks that are phenotypically the same, do not necessarily have the same genotype. Other studies also confirmed that the genetic correlation among Magelang and Tegal duck breeds was very near primarily based on microsatellite markers (Matitaputty et al. 2015; Susanti et al. 2021b).

Table 5. Particular alleles discovered at certain breeds of Central Javanese local duck

Breed	Specific allele	Size (bp)	Locus
Tegal Lemahan duck	C3	760	(AC)8CA
(ITP)	G3	526	(AC)8CA
	G4	158	(GA)8GG
Magelang duck (IM)	C5	572	(GA)8CC

Table 6. The average quantity of alleles according to breed of Central Javanese local duck

Breed of Central Javanese duck	Average number of alleles
Magelang duck (IM)	2.84
Peking duck (IK)	3.12
Pengging duck (IG)	2.52
Tegal Branjangan duck (ITB)	2.68
Tegal Jarakan duck (ITJ)	2.52
Tegal Blorong duck (ITL)	2.64
Tegal Lemahan duck (ITP)	2.88

The data ISSR marker based presented here are important records for confirming the genetic archive of the domestic duck population. ISSR is considerably utilized in the confirmation of genetic association between populations in numerous living organisms such as Japanese quail (*Coturnix japonica*) (Eissa et al. 2014), silkworm (*Bombyx mori*) (Bakkappa et al. 2011), Anatolian duck (Tunca et al. 2015), rabbit (El-Sabrout and Aggag 2015), camel (*Camelus*) (Barazandeh et al. 2020), and *G. g. domesticus* (Silva et al. 2020). The documentation on relationship and genetic archive of duck populations has important meaning regarding genetic refinement and proposed action of duck breeding in Indonesia in the future. In animal genetic diversity control, one prime concern is genetic linkage among native livestock populations. The genetic study of the population of wild and domestic ducks in Indonesia is very limited. This research contributes for study of genetic diversity and genome evolution of Central Javanese ducks in the future. Further research related to the genetic archive of wild and domestic duck populations in Indonesia is urgently needed as a source of genetic substance for Indonesian germplasm. A comprehensive understanding of the potential of the native species of Central Java ducks is urgently needed to support long-term genetic improvement of the ducks. The ISSR marker was found to be quite efficient in distinguishing every genotype at the molecular degree and might be utilized for genetic variety investigation of animals.

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